HISTOPATHOLOGICAL STUDIES OF PINE ROOTS INFECTED WITH LANCE AND PINE CYSTOID NEMATODES

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SUMMARY

Histopathological studies were made of slash and loblolly pine roots infected by 2 endoparasitic species of nematodes, *Hoplolaimus coronatus*, the lance nematode; and *Meloidodera floridensis*, the pine cystoid nematode. Both species cause severe damage to pine roots in southeastern North Carolina. Lance nematodes penetrated both lateral and mycorrhizal short roots; by feeding on and migrating through the cortex, they caused extensive internal damage. In several cases, these nematodes were in the cortex with their heads curved toward the vascular tissue. Pine cystoid nematode larvae also entered both lateral roots and mycorrhizae. Mature females developed in the cortex, in some cases breaking the outer layer of tissue as their bodies enlarged. Giant cells surrounded by hyperplastic tissue developed in the region adjacent to the head of the maturing female. Maturation and enlargement of the female nematodes caused, in certain instances, a compression and collapse of the surrounding tissue.

The relationships of certain plant-parasitic nematodes to the stunting of pines in outplantings have been reported (7). Four predominant species *Helicotylenchus nannus* Steiner, 1945; *Xiphinema americanum* Cobb, 1913; *Hoplolaimus coronatus* Daday, 1905; and *Meloidodera floridensis* Chitwood, *et al.*, 1956, were the most important nematode pathogens. In greenhouse tests, the latter 2 species were the most destructive, as evidenced by reductions in top growth and root damage. This paper reports results of a histopathological study of pine roots infected with lance nematodes, *H. coronatus*, and pine cystoid nematodes, *M. floridensis*. A preliminary report has been given (6).

MATERIALS AND METHODS.—Root systems of slash pine (*Pinus elliottii* Engelm.) and loblolly pine (*P. taeda* L.) seedlings infected with lance or cystoid nematodes were selected for study. The roots were washed carefully with a fine spray of water to remove all soil and were fixed in FAA for 24 hours (8). Samples of branch roots taken from the fixed pine root systems were dehydrated through a tertiary butyl alcohol series and embedded in tissue-mat (melting point, 54-56°C). Sections 10-12 μ thick were stained with safranin and fast green and mounted in Permount synthetic resin. Serial sections of slash and loblolly roots infected with lance nematodes and another group infected with pine cystoid nematodes were studied.

EXPERIMENTAL RESULTS.—*H. coronatus*.—Larvae and adults of the lance nematode penetrated both lateral roots and mycorrhizae of both slash and loblolly pine but remained in the cortex which was infected with mycorrhizal fungi. Migration occurred through this tissue in a path parallel to the long axis of the root, resulting in considerable cell damage, mostly restricted to the area immediately around the nematode (Fig. 1-A). Cells in the path of the

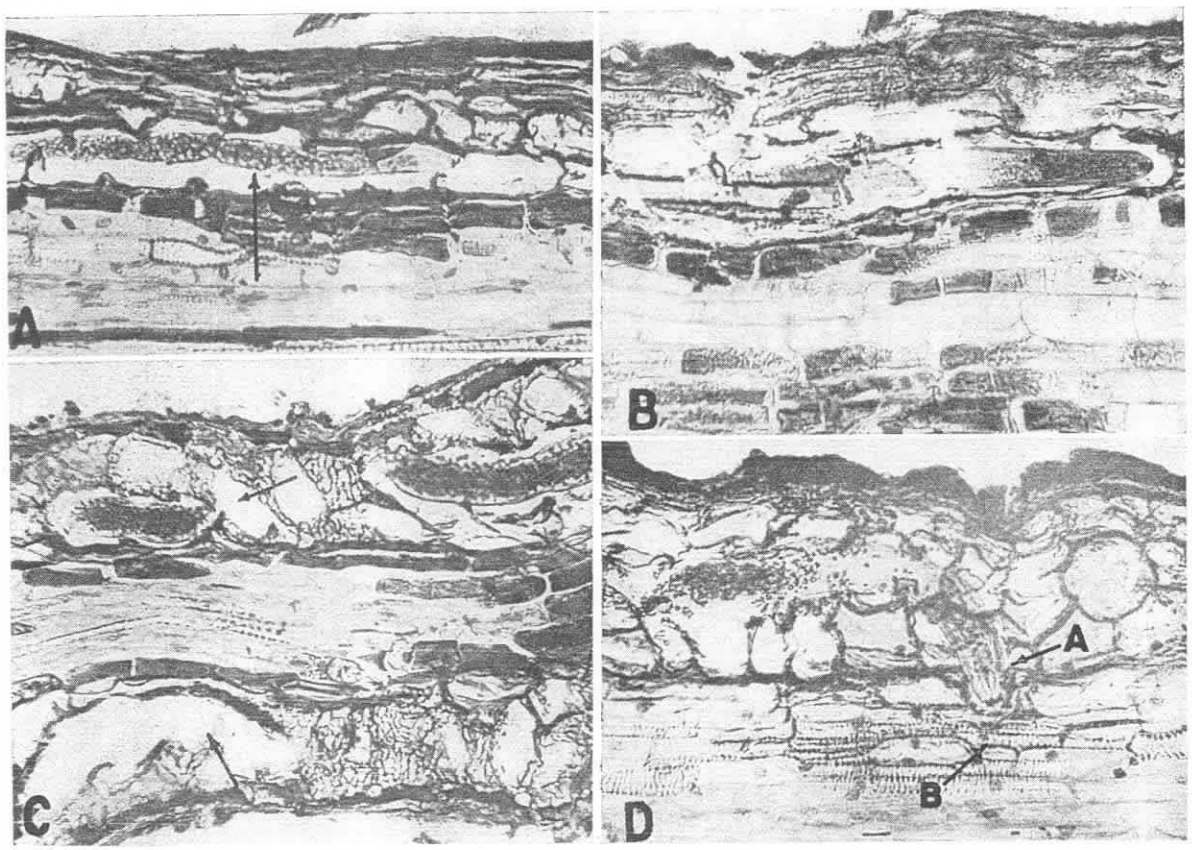

Fig. 1. Photomicrographs of lance nematode injury in pine root: **A)** Larva in the region of cortex infected with mycorrhizal fungi lying parallel to the long axis of the lateral root (arrow). **B)** Cortical cells of lateral root collapsed in path of nematode. **C)** Cortical cells of mycorrhiza damaged by migrating nematodes (arrows). **D)** Lance nematode in the cortex of a lateral root with its head turned toward the vascular elements: **a)** head of nematode, **b)** vascular tissue. (× 190).

invading nematode collapsed, in many cases forming a passageway just large enough for the movement of the nematode (Fig. 1-B). Considerable damage was caused by the migration of the nematodes through the section of cortex infected with mycorrhizal fungi of the mycorrhizae (Fig. 1-C). In certain short roots, 1-2 cell layers on either side of the invading nematode took a red stain rather than green (as noted for healthy cortical cells). Nuclei of these cells stained reddish-brown and entire cells appeared devoid of protoplasm. The walls of these cells also were thicker than those of nonaffected cells, as noted by Krusberg and Sasser (5) in cotton roots infected by lance nematodes. In several instances, a nematode was found in the cortex with its head curved toward the vascular tissue (Fig. 1-D). Cells of the xylem were punctured by the stylet of the feeding nematode. Considerable disruption and collapse of cells were evident at the feeding loci.

M. floridensis.—Larvae of the pine cystoid nematode entered lateral roots and mycorrhizae of both slash and loblolly pine through the area behind the root tips, fissures in the outer root layers (particularly those made by side roots erupting the outer layers during their development), and wounds. Destruction of cells by larvae entering the roots was slight. Migration through root tissues was largely intercellular. Swollen females were generally found in the cortex (Fig. 2-A), in some cases bursting the outer layer of tissue exposing part of the pearl-white body. The heads of most females were oriented toward the proximal end of the root both in the undifferentiated meristematic tissue behind the tips of mycorrhizae and in the region of cortex not infected with mycorrhizal fungi of small lateral roots. Giant cells adjacent to the head of the female nematode developed in the region of cortex not infected with mycorrhizal fungi, in protophloem, and in protoxylem in both slash and loblolly pine roots (Fig. 2-B). Giant cells were found not uncommonly in the differentiated tissues behind the root tips of the mycorrhizae. The part of the giant cell wall in contact with the head of the female nematode was thicker than the remaining wall area. Hyperplasia of the cortical and vascular parenchyma cells surrounding the giant cells was very common. In several instances, the development of the giant cell and

enlargement of the female caused a disruption and thrusting aside of the vascular elements. Tracheids passed around giant cells, somewhat pushed out of position, but their continuity was unbroken. The cells adjacent to the swollen female were collapsed and compressed and the cell layers surrounding these collapsed cells held their shape, but were filled with phlobaphenes (Fig. 2-B). Some larvae were found embedded in the tissue surrounding the cavity produced by the mature female and were considered

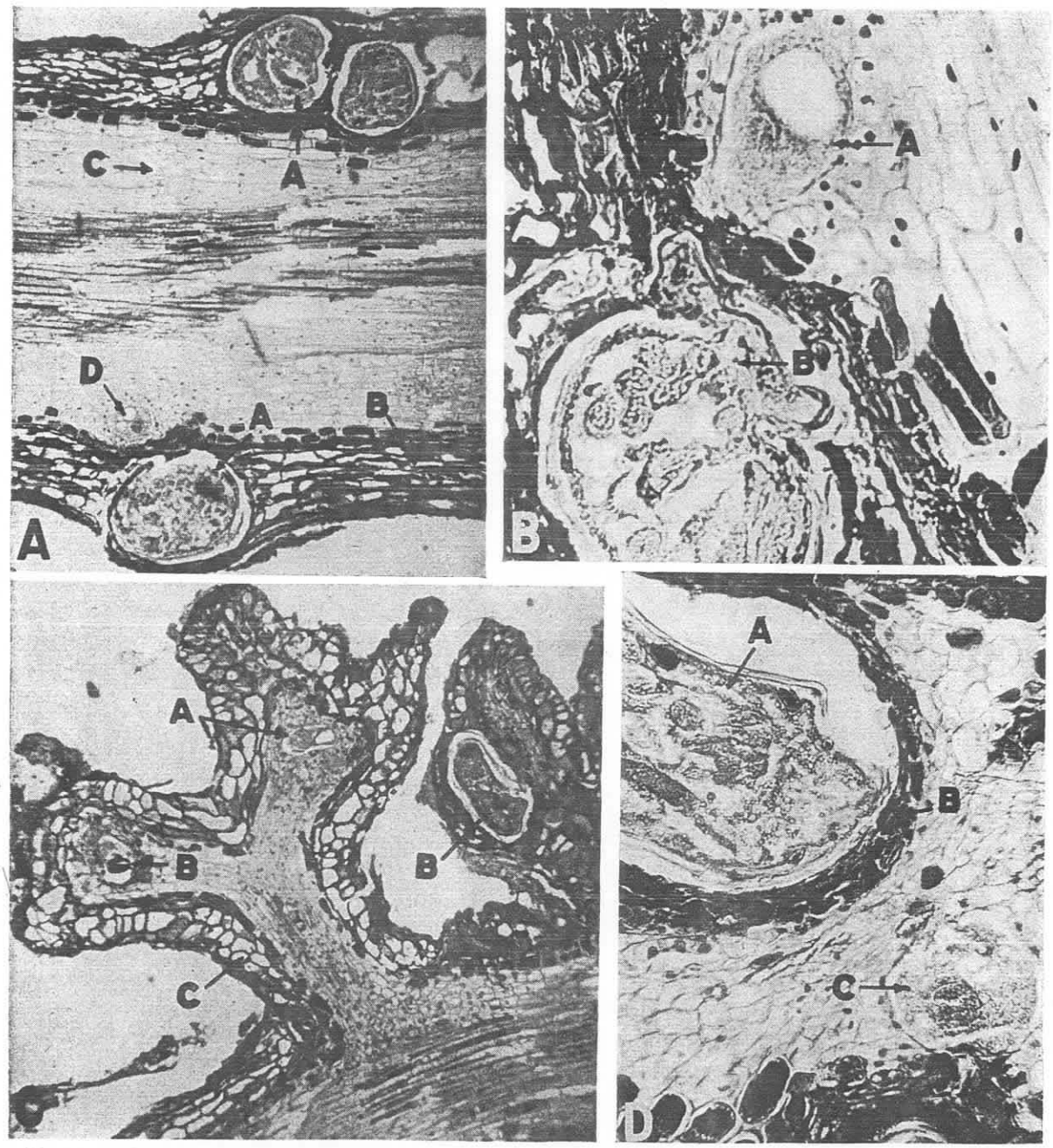

Fig. 2. **A)** Female nematodes in the region of cortex infected with mycorrhizal fungi of a lateral root of pine: **a)** nematodes, **b)** mycorrhizal-fungus-infected cortex, **c)** nonmycorrhizal-fungus-infected cortex, and **d)** giant cell (× 50). **B)** Giant cell adjacent to the head of a female nematode: **a)** giant cell in the nonmycorrhizal-fungus-infected cortex surrounded by hyperplastic tissue, **b)** female nematode in the mycorrhizal-fungus-infected cortex (× 180). **C)** Giant cell in the meristematic tissue behind the tip of a mycorrhiza: **a)** giant cell, **b)** nematodes, and **c)** mycorrhizal-fungus-infected cortex (× 50). **D)** Cross section of a small lateral root showing the compression and collapse of cells adjacent to the maturing female: **a)** female nematode, **b)** red-stained cells filled with phlobaphenes, and **c)** giant cell (× 190).

second generation larvae which had not escaped from the root after hatching. Mature males were not found in any of the roots examined.

DISCUSSION AND CONCLUSIONS.—These investigations have shown that lance and pine cystoid nematodes infect and severely damage the roots of both slash and loblolly pine. The lance nematode feeds primarily as an endoparasite in both lateral roots and mycorrhizae. This endoparasitic feeding habit agrees with the findings of Krusberg and Sasser (5) in cotton, with the exception that rather than feeding mostly on phloem parenchyma and phloem elements as noted in cotton, this nematode is usually restricted to the cortex in slash and loblolly pine roots. Instances were noted, however, in which the head of the nematode lying in the cortex was curved toward the vascular elements. Necrosis of cells not immediately in contact with the nematode is believed caused by the secretions emanating from the stylet which have diffused ahead of the nematode. Christie (2), in discussing the various kinds of injury that nematodes inflict on plants, stated ". . . I believe that most of the injury that nematodes inflict on the higher plants is biochemical and that the source of the secretions causing this injury are (*sic*) the nematode's salivary or esophageal glands." This type of tissue damage could, in part, explain the sloughing off of the cortex of the lateral roots and mycorrhizae.

The giant cells formed in pine roots by *Meloidodera* were found either in the cortex of lateral roots or in undifferentiated tissue in the meristematic regions in the tips of mycorrhizae, and not within the cells of the vascular bundles as in *Meloidogyne* and *Heterodera* (1, 3, 4, 9). This type of host-parasite relationship might be more balanced than one in which giant cells are formed in the vascular tissue where translocation of water and nutrients are probably impaired. Further evidence supporting this balanced relationship between *Meloidodera* and pine is the absence of root galls and the fact that extremely high populations are necessary for appreciable damage to pine.

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